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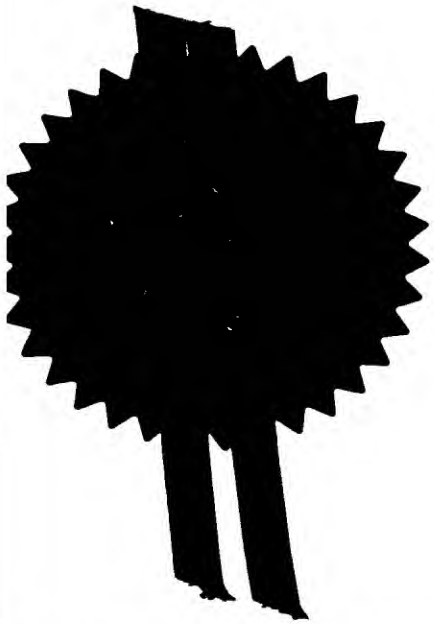

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Dated 02 OCT 2000

Patents Form 1/77

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Request for grant of a patent

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17 SEP 1999

The Patent Office
Cardiff Road
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1. Your reference HOLMS 9999

2. Patent application number *(The Patent Office will fill in this part)* **9921881.0**

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*
RUPERT HOLMS,
PRIMROSE HILL, 66 REGENT'S PARK ROAD, LONDON NW1 7SX.

THE PATENT OFFICE
17 SEP 1999
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Patents ADP number *(if you know it)* 6666530

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention
REGULATORY/UNFOLDING PEPTIDES OF EZRIN

5. Name of your agent *(if you have one)* No

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*
RUPERT HOLMS
PRIMROSE HILL
66 REGENT'S PARK ROAD
LONDON NW1 7SX

Patents ADP number *(if you know it)*

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

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Number of earlier application	Date of filing *(day / month / year)*

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b) there is an inventor who is not named as an applicant, or
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Description	40 (INC ABSTRACT & CLAIMS) 36
Claim(s)	2
Abstract	2
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Priority documents	No
Translations of priority documents	No
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	No
Request for preliminary examination and search *(Patents Form 9/77)*	Yes 1
Request for substantive examination *(Patents Form 10/77)*	No
Any other documents *(please specify)*	No

11. I/We request the grant of a patent on the basis of this application.

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DUPLICATE

Application for a Patent in the United Kingdom

Title:

Regulatory/Unfolding Peptides of Ezrin

INVENTOR AND APPLICANT

Rupert Holms Ph.D.

Address for correspondence:

Dr Rupert Holms,
66 Regent's Park Road,
London NW1 7SX
Telephone: (44) 171 722 9796
Fax: (44) 171 722 8115
Email: rupertholm@aol.com

PRIORITY DOCUMENTS

None

PATENT DOCUMENTS

United States Patent 5,773,573
Rupert Holms 30th June 1998

OTHER PUBLICATIONS

Ossi Turunen, Markku Sainio, Juha Jaaskelainen, Olli Carpen, Antti Vaheri (1998)
"Structure – Function relationships in the ezrin family and the effect of tumor-associated point mutations in neurofibromatosis 2 protein" *Biochimica et Biophysica Acta* **1387:** 1-16

Anthony Bretscher, David Reczek and Mark Berryman (1997)
"Ezrin: a protein requiring conformational activation to link microfilaments to the plasma membrane in the assembly of cell surface structures" *Journal of Cell Science* **110**: 3011-3018

Claudia Hecker, Christoph Weise, Jurgen Schneider-Schaulies, Harvey Holmes, Volker ter Meulen (1997) " Specific binding of HIV-1 envelope protein gp120 to the structural membrane proteins ezrin and moesin." *Virus Research* **49:** 215-223

M Levancher, F Hulstaert, S. Tallet, S Ullery, J J Pocidalo, B A Bach (1992)
"The significance of activation markers on CD8 lymphocytes in human immunodeficiency syndrome: staging and prognostic value" *Clinical Experimental Immunology* **90** 376-382

Stephan Oehen and Karin Brduscha-Riem (1998)
"Differentiation of Naïve CTL to Effector and Memory CTL: Correlation of Effector Function with Phenotype and Cell Division" *The Journal of Immunology* **161** 5338-5346

Regulatory/Unfolding Peptides of Ezrin

BACKGROUND TO THE INVENTION

The field of the present invention relates to the treatment of infectious disease and cancer by inducing disease fighting immune responses. The growing problem of new strains of pathogenic bacteria resistant to antibiotics, the limited range of compounds effective against chronic viral and fungal infections and shortage of effective anti-cancer treatments demonstrates the need for compounds that can enhance the host defence against these medical problems. This invention relates to novel charged molecules which stimulate immune responses by binding to the Hepreceptor, a novel active site in human ezrin which I have discovered. The preferred charged molecules are novel peptides with sequences identical to the Hepreceptor in human ezrin.

Ezrin is a member of the ERM (ezrin-radixin-moesin) family of proteins which play structural and regulatory roles in a wide range of cell types. There is considerable evidence to indicate that ezrin regulates the structure of the cortical cytoskeleton to control cell surface topography. Ezrin adopts two main conformations: 1) a soluble folded form which is found in the cytoplasm and, 2) an unfolded and elongated form which is found attached to the cytoplasmic surface of the cell membrane particularly in conjuction with microvilli and other activation related structures. The N terminal half of the protein is attached to the cytoplasmic surface of the membrane while the C terminal part binds to the actin cytoskeleton. Ezrin is a tyrosine kinase substrate in T cells and is also tyrosine phosphorylated as a result of Epidermal Growth Factor (EGF) stimulation of the EGF receptor. The N terminal Domain of ezrin in its extended conformation binds to the cytoplasmic tail of CD44 in the presence of PIP_2. Ezrin also may bind to the cytoplasmic tail of ICAM-2. Ezrin is very sensitive to regulatory proteases such as calpain and is rapidly turned over during cell activation.

Anthony Bretscher, David Reczek and Mark Berryman (1997)
"Ezrin: a protein requiring conformational activation to link microfilaments to the plasma membrane in the assembly of cell surface structures" *Journal of Cell Science* **110**: 3011-3018

Detailed analysis of the secondary structure of ezrin shows that there are three main structural domains: an N terminal domain from amino acids 1 to 300, a highly charged alpha domain from amino acids 300 to 470 and C terminal domain from amino acids 470 to 585. Structural modelling suggests that the alpha domain is folded into two anti-parallel helices in the soluble globular form of ezrin although the location of the hinge has not been identified. In the model of the extended phosphorylated form, ezrin is attached to the inner surface of the cell membrane by the N terminal domain, the alpha domains of two ezrin molecules are paired into anti-parallel dimers and located below the cell surface membrane. In this extended form, ezrin is tyrosine phosphorylated at tyrosine 353 (Yp 353).

Ossi Turunen, Markku Sainio, Juha Jaaskelainen, Olli Carpen, Antti Vaheri (1998) "Structure – Function relationships in the ezrin family and the effect of tumor-associated point mutations in neurofibromatosis 2 protein" *Biochimica et Biophysica Acta* **1387**: 1-16

I disclosed in United States Patent 5,773,573 that the fourteen amino acid peptide HEP1, (amino acid sequence of TEKKRRETEREKE, SEQ ID 28, identical to amino acids 324-337 of human ezrin) which has a 70% identity to the C terminus of gp120 could inhibit HIV replication in vivo in man. At the time I believed that the observed anti-HIV effect of peptide HEP1 was due to the orally administered HEP1 inducing

immunological tolerance to an auto-reactive immune response induced by the complementary HIV sequence at the C terminus of gp120.

I have now discovered that the anti-HIV activity of HEP1 is due to the activation of a novel immunological pathway in which HEP1 binds to its complementary sequence in Domain B of the Hepreceptor (HEP1 is identical to part of Domain A of the Hepreceptor).

SUMMARY OF INVENTION

This invention describes a novel regulatory site in human ezrin (the Hepreceptor) and charged molecules which bind to this site to induce immune responses. I have determined that the Hepreceptor (spanning amino acids 308-373 of human ezrin) in the soluble conformation of ezrin comprises of two adjacent alpha helical Domains which are folded together at a hinge region (M339-M340) into two anti-parallel helices stabilised by complimentary side chain charges of the primary amino acid sequence. (see TABLE 1 and FIGURE 1). I have determined that in the unfolded membrane associated conformation of ezrin, the Hepreceptor is pushed through the cell membrane and is exposed on the outer surface of the cell (see FIGURE 2). This unexpected discovery is in contrast to models of membrane organisation in scientific publications to date, which do not show any part of the alpha domain of ezrin on the outer cell surface. It is my invention that peptides with amino acid sequences identical to parts of the Hepreceptor bind to complementary parts of the Hepreceptor which results in medically useful immunological responses. It is also my invention that any charged molecule binding to this site will induce medically useful immune responses. These charged molecules can be administered orally and by other routes for the treatment of various infectious diseases and cancer.

TABLE 1 Amino acids, three letter code, one letter code and side chain charges

CHARGES ON AMINO ACID SIDE CHAINS AT PHYSIOLOGICAL pH				
Amino acid	**Three letter code**	**One letter code**	**Charge**	**Symbol**
Glycine	Gly	G	NONE	
Alanine	Ala	A	NONE	
Valine	Val	V	NONE	
Isoleucine	Ile	I	NONE	
Leucine	Leu	L	NONE	
Serine	Ser	S	NONE	
Threonine	Thr	T	NONE	
Aspartic acid	Asp	D	NEGATIVE	--
Glutamic acid	Glu	E	NEGATIVE	--
Phosphotyrosine	Tyr(P)	Yp	NEGATIVE	--
Asparagine	Asn	N	WEAK NEGATIVE	-
Glutamine	Gln	Q	WEAK NEGATIVE	-
Lysine	Lys	K	POSITIVE	+
Arginine	Arg	R	POSITIVE	+
Histidine	His	H	WEAK POSITIVE	+
Proline	Pro	P	NONE	
Tryptophan	Trp	W	NONE	
Phenylalanine	Phe	F	NONE	
Tyrosine	Tyr	Y	NONE	
Methionine	Met	M	NONE	
Cysteine	Cys	C	NONE	

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a diagram of the alignments of the primary amino acid sequences of:

a) the folded anti-parallel associated helices of the Hepreceptor in soluble ezrin.
b) the unfolded helix of the Hepreceptor in membrane associated ezrin with an example of a peptide ligand.
c) two unfolded Hepreceptors forming a dimer of anti-parallel associated helices during an interaction between two cells.

FIGURE 2 is an illustration of the relationship between the Hepreceptor on ezrin, its ligands, cell membranes, cell surface receptors and cytoskeletal components.

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID 1 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 2 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 3 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 4 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 5 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 6 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 7 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 8 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 9 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 10 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 11 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 12 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 13 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 14 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 15 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 16 is an amino acid sequence of a peptide according to the present invention
SEQ ID 17 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 18 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 19 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 20 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 21 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 22 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 23 is an amino acid sequence of a peptide according to the present invention.

SEQ ID 24 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 25 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 26 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 27 is an amino acid sequence of a peptide according to the present invention.
SEQ ID 28 is an amino acid sequence of a peptide according to the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The soluble conformation of ezrin found in the cytoplasm comprises of two adjacent alpha helical domains which are folded together at a hinge region (M339-M340) into two anti-parallel helices stabilised by complimentary side chain charges of the primary amino acid sequence. It is the subject of this invention that the positively and negatively charged side chains of the amino acid sequence of Hepreceptor Domain A are complementary to the positively and negatively charged side chains of the amino acid sequence of the Hepreceptor Domain B. In the activated open conformation of ezrin, the interaction of the Domain A and Domain B of the Hepreceptors of two different ezrin molecules allows the formation of anti-parallel dimers. In addition to the antiparallel dimers of ezrin which form below the cell surface, I have determined that these dimers can form between a Hepreceptor exposed on the surface on one cell with a Hepreceptor exposed on the surface of another cell. When the two Hepreceptors make contact during close association of two cell surfaces an activation signal is initiated in both cells (FIGURE 2). Any charged molecule that partially mimics the interaction between the side chains charges of Domain A and Domain B of the Hepreceptors will give rise to a medically useful biological response.

Hepreceptor-Domain A (amino acid numbers 308-339 of human ezrin), comprises of the following 32 amino acid sequence.

(The sequences are listed using the single letter code for each amino acid written from the N terminus to the C terminus of the polypeptide. Y'p represents the form of phosphotyrosine found in vivo)

SEQ ID 1

A R E E K H Q K Q L E R Q Q L E T E K K R R E T V E R E K E Q M

In United States Patent 5,773,573, I disclosed the anti-HIV activity of peptide HEP1 (SEQ ID 28) which I have now discovered has a sequence identical to part of Hepreceptor-Domain A (spanning amino acids 324-337 of the human ezrin sequence). In the above patent I made the assumption that anti-HIV activity was due to the induction of immunological tolerance to autoreactive immune responses induced by the C terminus of HIV gp120. I can now disclose that the anti-HIV activity of HEP1 is due to its binding to Hepreceptor Domain B and the induction of a novel immune response. It is a subject of this invention that there are novel peptides derived from the Hepreceptor of ezrin with significantly superior activity to HEP1.

Hepreceptor-Domain B (amino acid numbers 340-373 of human ezrin), comprises of the following 34 amino acid sequence (Tyrosine 353 [Y] may be phosphorylated to phosphotyrosine [Yp] in the membrane associated conformation of ezrin):

SEQ ID 2

M R E K E E L M L R L Q D $Y_{(p)}$ E E K T K K A E R E L S E Q I Q R A L Q

I have determined that Domain B of the Hepreceptor is the site on ezrin to which HIV gp120 binds during infection of the brain. (HIV gp120 binds to Hepreceptor Domain B using its charged C terminal amino acids which have a 70% identity to part of Hepreceptor Domain A). Novel charged molecules which bind to the Hepreceptor may be useful in treating HIV related dementia.

Claudia Hecker, Christoph Weise, Jurgen Schneider-Schaulies, Harvey Holmes, Volker ter Meulen (1997) " Specific binding of HIV-1 envelope protein gp120 to the structural membrane proteins ezrin and moesin." Virus Research **49** 215-223

I have demonstrated (EXAMPLE 1) that HEP1 therapy (10mg per day orally either for thirty days or ninety days) in 21 HIV infected patients induces immune responses which leads to clinical improvement over the following six months after therapy, as measured by an increasing CD4 T lymphocyte population and declining opportunistic infections, declining HIV infectivity and declining CD38,CD8 population of T lymphocytes (an established prognostic marker of the progression to AIDS).
M Levancher, F Hulstaert, S. Tallet, S Ullery, J J Pocidalo, B A Bach (1992)
"The significance of activation markers on CD8 lymphocytes in human immunodeficiency syndrome: staging and prognostic value" *Clinical Experimental Immunology* **90** 376-382

A mean increase in the level of expression of CD44 and MHC Class I on T lymphocytes over six months was observed which appears to also correlate with the clinical improvement. No toxicity was detected with the administration of HEP1. Increases of MHC Class I expression and CD44 expression are associated with increases in memory T cells and Class I restricted cell mediated immunity.
Stephan Oehen and Karin Brduscha-Riem (1998)
"Differentiation of Naïve CTL to Effector and Memory CTL: Correlation of Effector Function with Phenotype and Cell Division" *The Journal of Immunology* **161** 5338-5346

The results of this trial demonstrates that a peptide or other charged molecule which mimics all or part of the Hepreceptor can give rise to an activation signal that eventually leads to a change in the homoeostasis of the immune system and long term up regulation of cell mediated and humoral immunity. I have also demonstrated that acute and chronic candida infection in women can be treated and cured by the immune response arising from Hepreceptor stimulation (EXAMPLE 2). I have demonstrated that peptides derived from the Hepreceptor can activate monocytes and macrophages in mice both in vitro and in vivo, which leads to a protective immune response. (EXAMPLE 3). Peptides of this invention have a significantly higher activity than HEP1. Hepreceptor stimulation also leads to the activation of human peripheral blood mononuclear cells which was demonstrated by measuring the incorporation of radioactive tritiated thymidine into DNA of the growing cells. Novel peptides, Rupe312 and Rupe414 derived form Hepreceptor Domain A had a ten fold higher activity than HEP1. (EXAMPLE 4)
(Rupe312 SEQ ID 8:KKRRETVERE and Rupe414 SEQ ID 13: KRRETVEREKE)

I also discovered that a 24 hour incubation of human White Blood Corpuscles (WBC) with peptides derived from the Hepreceptor results in a fall in MHC Class I cell surface expression, probably due to cell activation and receptor internalisation, and an increase in the total population of macrophages expressing MHC Class I. This is consistent with the long term increase in the population cells expressing MHC Class I seen in HIV patients during the six months following HEP1 therapy. In this assay system Rupe312 and Rupe414 had significantly higher activity than HEP1 (EXAMPLE 5).
(Rupe312 SEQ ID 8:KKRRETVERE and Rupe414 SEQ ID 13: KRRETVEREKE)

This invention describes charged molecules which specifically bind to the Hepreceptor. I have designed three groups of novel charged peptides which have sequences identical to the amino acid sequences of the complementary domains of the Hepreceptor and which either bind to Hepreceptor Domain B (SEQ ID 3 - SEQ ID 16), or to both Domain A and B (SEQ ID 17), or which bind to Domain A (SEQ ID 18 - SEQ ID 27). The peptides which are a subject of this invention probably bind to

cell surface exposed Hepreceptors and stabilise the unfolded conformation of ezrin and induce immuno-modulatory effects. The preferred peptides are between five and thirteen amino acids in length and the preferred sequences are as follows.

Hepreceptor Domain B binding peptides:

SEQ ID 3
Rupe15: TEKKR

SEQ ID 4
Rupe19: TEKKRRETV

SEQ ID 5
Rupe111: TEKKRRETVER

SEQ ID 6
Rupe37: KKRRE

SEQ ID 7
Rupe310: KKRRETVE

SEQ ID 8
Rupe312: KKRRETVERE

SEQ ID 9
Rupe313: KKRRETVEREK

SEQ ID 10
Rupe314: KKRRETVEREKE

SEQ ID 11
Rupe411: KRRETVER

SEQ ID 12
Rupe413: KRRETVEREK

SEQ ID 13
Rupe414: KRRETVEREKE

SEQ ID 14
Rupe59: RRETV

SEQ ID 15
Rupe614: RETVEREKE

SEQ ID 16
Rupe1014: EREKE

Hepreceptor Domain A and Domain B binding peptide:

SEQ ID 17
Rupe1024 EREKEQMMREKEEL

Hepreceptor Domain A binding peptide:

SEQ ID 18 Rupe2024:	KEELM
SEQ ID 19 Rupe2032:	KEELMLRLQDYEE
SEQ ID 20 Rupe2032p:	KEELMLRLQDYpEE
SEQ ID 21 Rupe2132:	EELMLRLQDYEE
SEQ ID 22 Rupe2132p:	EELMLRLQDYpEE
SEQ ID 23 Rupe2232:	ELMLRLQDYEE
SEQ ID 24 Rupe2232p:	ELMLRLQDYpEE
SEQ ID 25 Rupe2428:	MLRLQ
SEQ ID 26 Rupe2832:	QDYEE
SEQ ID 27 Rupe2832p:	QDYpEE

Other peptides or other charged molecules which bind to Domain A or Domain B or bridge Domain A and Domain B of the Hepreceptor are likely to be biologically active. These peptides or other charged molecules can be administered orally and by other routes for the treatment of various infectious diseases and cancer.

HOW TO MAKE

Peptides used in this invention may be synthesised for example, using a solid phase method using either Boc or Fmoc chemistry or any other practical route for peptide synthesis known to those skilled in the art of peptide synthesis.

Stepwise solid phase synthesis with Boc-amino acids can be performed based on the method of Merrifield; (Journal of American Chemical Society 85 2149-2154). The following compounds can be used (Novabiochem resin: Boc-Glu(OBzl)-PAM, Amino acids: Boc-Lys (2Cl-Z-)OH, Boc-Glu(OBzl)OH, Boc-Arg(Tos)OH, Boc-Val-OH, Boc-Thr (Bzl)-OH, Solvents: DMF (Rathburn), Dichloromethane (BDH), Ethylacetate (BDH), Reagents: HBTU (Phase Separations Ltd), p-Cresol (Lanchester) TFA (Halocarbon Products Corporation) HF (BOC) DIEA (Fluka). Recommended reactive side chain protecting groups for Boc-amino acids are:, Arg (Tos), Asn (Xan), Asp (OcHxl), Glu (OcHxl) Gln (Xan) or Gln, His (DNP), Lys (CIZ), Serine (Bzl) Tyr (BrZ) Trp (CHO). The abbreviations have the following meanings: DCC= Dicyclohexylcarbodiimide, DIC=Diisopropylcarbodiimide, DCM=Dichloromethane, DMF=Dimethylformamide, TFA=Trifluoracetic acid, Boc= t-Butyloxycarbonyl, HOBT=Hydroxybenzotriazole, DIEA=Diisopropylethylamine, DCU=Dicyclohexylurea.

For example, boc synthesis of a peptide of this invention could be performed as follows: HBTU activation/in situ neutralization on 0.5 mmol scale uses 0.5 mmol of resin and a three fold excess of activated Boc amino acid. Boc amino acid and activating reagent (HBTU) should be used in equimolar quantities ie 2mmol each in this case equals a 3x excess. DIEA is used to both neutralise the resin for coupling and to activate the Boc-amino acid. (Hence 2.5 mmol is used, 1 equivalent Boc-aa and 1 equivalent resin). Reagents: 0.5M HBTU in DMF (MW=379, 0.5M=18.95g in 100ml, note it is not light stable) requires 2mmol=4ml and 2.5 mmol DIEA=0.46ml (MW=129, d=0.742). Activation of aminoacids: Boc-amino acid should be activated only immediately prior to addition to the resin, especially in the case of Arg (Tos). For all Boc amino acids: weigh 2 mmol Boc amino acid into a 20 ml glass sample bottle. Add 4ml 0.5M HBTU solution and shake to dissolve solid. Add 0.46 ml DIEA and mix (some colour change may be observed). Method: wash resin with DMF, remove Boc-protecting group with 100% TFA- Shake twice for 1 minute draining in between, drain, flow wash with DMF for 1 minute, drain, add activated amino acid solution, shake for 10 minutes, then take sample and perform the ninhydrin test to determin coupling efficiency. On completion of the synthesis flow wash with DMF, then DCM and dry. The synthesis of the first and every subsequent level of peptide construction is achieved using a three fold excess of HBTU activated Boc-amino acids in DMF. In all couplings, the coupling efficiency should be more than 99% as indicated by quantative ninhydrin testing. Deprotection of the N–termini is performed in 100% TFA. The resin peptide is carefully flow-washed before and after the deprotection. After the last coupling and removal of the Boc-protection, the peptide resin is washed with dichloromethane and dried by air. The peptide is removed from the resin support by the high HF method (2 g resin peptide, 2g cresol, 20ml HF, 1.5 h

at –5°C) to yield the crude peptide which is precipitated with ethylacetate (100ml) and redissolved in 6M guanidine HCL-0.1M TRIS solution (20ml).

The peptide can be purified as follows using an analytical HPLC separation on a Vydac C_{18} 5 RAC column. HPLC grade acetonitrile (aldrich) and water is filtered through a membrane filter and degassed with helium flow prior to use. Analytical separation achieved with a solvent gradient beginning with 0% acetonitrile, increasing constantly to 60% acetonitrile at 20 minutes, staying at this concentration for twenty minutes and decreasing steadily to 0% acetonitrile for 10 minutes at a constant flow of 1.2 ml per minute. Preparative separation of peptide achieved on a TSK-GEL preparative C_{18} column. Separation is achieved with a solvent gradient beginning with 0% acetonitrile, increasing constantly to 18% acetonitrile at 60 minutes, then 60% acetonitrile for 80 minutes, staying at this concentration for 30 minutes at a constant flow of 8 ml per minute. The gradient can be controlled by two microprocessor controlled Gilson 302 single piston pumps. Compounds can be detected with a Waters 486 Tunable Absorbance Detector at 214 nm and analytical chromatographs recorded with an HP laserjet 5L. A Holochrome UV-VIS detector 220 nm for preparative chromatographs can be recorded with an LKB 2210 single channel recorder. Capillary Electorphoresis quality control can be carried out using Waters Quanta 4000 equipment using a phosphate buffer (75 microM) pH2.5 run at 15 kV, sample time 20 seconds, loaded by hydostatic injection on 60 cm column, run time 12 minutes. The yield for 1 g 0.46 mmol resin synthesis should be about 250 mg pure peptide

Alternative solution synthesis methods may also be used to produce larger quantities of the peptides of this invention. Protected trimer fragments can be obtained using stepwise synthesis by the active esters method known to those skilled in the art of peptide synthesis. The fragments are then assembled using DCC/HOBT after removal of relevant C and N terminal protective groups. After removal of all protective groups the crude peptide is partially purified on SP-Sephadex-C25 ion exchange chromatography followed by preparative HPLC then lyophilised.

HOW TO USE

0.01 to 1000 mg of lyophilised peptide may be dissolved in 1-10 ml distilled water and administered orally or vaginally. 0.01 to 1000 mg may be formulated in to a pill or capsule or suppository with carriers used commonly by those skilled in the art of pill or capsule or suppository manufacture and administered orally or vaginally or anally. A filter sterilized solution of between 0.001 and 100 milligrams of peptide in distilled water may be injected subcutaneously or intravenously or intramuscularly.

The following Examples serve to illustrate the invention only, and should not be construed as limiting it in any way.

EXAMPLE 1

HEP1 therapy (10mg per day orally either for thirty days or ninety days) in 21 HIV infected patients lead to clinical improvement. The success of this study demonstrates generally the utility and reduction to practice of peptides derived from the Hepreceptor.

This study was performed with pharmaceutical grade HEP1, a peptide which has an identical sequence to part of Domain A of the Hepreceptor. HIV-infected volunteers were recruited for the study at the Institute of Immunology, Moscow under the guidance of Professor Ravshan Ataullakhanov. The pharmaceutical grade HEP1 passed an extensive range of animal (rat and rabbit) toxicology and pre-clinical testing before the trial commenced, which demonstrated the safety of the compound. (Preliminary evaluation of toxicity- negative , Effect of 1000x therapeutic dose-negative, Local Irritation- negative, Influence on CNS and HVS-negative, Sub-acute toxicity-negative, Mutagenic effects-negative, Chronic toxicity-negative, Embryotoxicity- negative)

Study plan

Patients were orally administered a solution of 10mg of HEP1 in 2ml sterile distilled water once a day in the morning before breakfast (the solution was prepared and stored is separate 10mg lots at $-20^{\circ}C$). All patients were administered a coded placebo solution of distilled water for thirty days before treatment. A first group of 11 patients were administered HEP1 for 90 days and a second group of 10 patients were administered HEP1 for 30 days fifteen months later, after the data from the first group of patients had been analysed. During the treatment period the patients were requested to attend the clinic once a week, undergo a medical examination and give a blood sample for analysis. The patients were also requested to co-operate with post treatment monitoring and attend the clinic once a month for six months for further medical examinations and donations of blood samples. 21 out of 21 patients co-operated with monitoring during the treatment period and 14 out of 21 patients agreed to post treatment monitoring. The patients were not receiving any other anti-retroviral therapy during or one month before HEP1 treatment.

Patients

Patients were recruited from various clinics around Moscow and gave informed written consent to participate in the trial. They were identified as HIV infected by a positive ELISA assay, had depressed T cell counts and experiencing some clinical manifestation of HIV related illness. The patients were subsequently shown to have a range of CD4 cells per microlitre between 17 and 801 and a range of serum HIV RNA (Roche Labs Amplicor quantitative PCR assay) from undetectable to 230,000 copies per ml.

Patient characteristics at start of trial

ID Code	Sex	Age	Est Period of infection	CD4 cells / microL	HIV RNA Copies / ml	opportunistic infections	other
P1	male	45	8	219	500	severe	
P2	male	33	2	192	10000	severe	
P3	female	45	6	481	2000	severe	
P4	female	16	8	237	43000	severe	
P5	male	27	2	123	<400	moderate	very sick
P6	female	23	1	357	10000	moderate	Herpes z
P7	male	23	8	139	94000	very low	Herpes z
P8	female	38	10	320	22000	very low	Ovarian cyst
P9	male	43	3	17	10000	severe	
P10	male	19	2	155	21000	severe	On opiates
P11	male	35	3	188	<400	severe	Active TB
P12	male	32	8	175	13000	severe	
P17	male	25	1	478	11000	severe	
P21	female	37	10	98	11000	severe	
P63	male	35	1	651	4000	severe	
P67	female	31	7	124	230000	severe	Very sick
P68	female	51	2	597	<400	severe	Very sick
P69	male	34	1	192	8000	severe	Active TB
P72	male	33	1	534	25000	severe	Active TB
P73	male	30	1	801	<400	severe	Active TB
P76	female	38	7	72	9000	severe	

General observations

The patients reported no adverse reactions to HEP1, and 17 patients reported they felt generally better and gained at between 1.5Kg and 4.5Kg in weight while on HEP1 therapy (one patient also felt better during the placebo period).

Adverse reactions

No adverse reactions were detected. Clinical assessments including ultrasound examinations and an extensive series of biochemical, haematological, immunological blood tests and urine tests were performed.

Opportunistic infections

Opportunistic infections were detected by microbiological analysis and patients treated by HEP1 were either stable with no new infections or infections declined. For example, before treatment 38% of the patients had severe Candida Ablicans infection of the pharynx, after treatment only 9% were severely infected. Before treatment, 52% of the patients had severe S viridans infection of the pharynx, after treatment only 33% were severely infected. Before treatment, 33 % of the patients had S aureus infection of the pharynx, after treatment only 19 % were infected.

CD4 T lymphocytes

The group eleven patients were treated for three months with HEP1 experienced an average gain of T cell numbers of 9% by the end of treatment and 32% on average over the following six months after treatment. The group ten patients who were treated for one month with HEP1 experienced an average gain of T cell numbers of 3% by the end of treatment and 20% on average over the following six months after treatment. The continued improvement suggests that some positive immunological change had been induced in the patients.

HIV infectivity assay by TCID

Viral load was measured by mixing HIV infected Peripheral Blood Mononuclear Cells (PBMC) from the patient with uninfected donor PBMC in a ratio between sample cells to culture cells of 1/16 and culturing at 37°C for fourteen days. Culture viral load was measured by an Innogenetics HIVp24 assay and the results were recorded in picograms of HIVp24 antigen per ml.

Three month treatment

Patient	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
Infectivity in pg/ml p24max at start of trial	1039	315	na	386	515	203	1113	369	1074	na	480
re-based to 100 at start	100	100	0	100	100	100	100	100	100	0	100
Phase O average						130					
Phase 1 average	7	32		0	28	0	47	0	39		
Phase 2 average	21	218		198	129	251	70	217	40		835
Phase 3 average	0	61		0	85	0	48		24		

One month treatment

Patient	P12	P17	P21	P63	P67	P68	P69	P72	P73	P76
Infectivity in pg/ml p24max at start of trial	465	0.3	3.1	2.9	6642	6.5	3.2	2	1	4.5
re-based to 100 at start	100	100	100	100	100	100	100	100	100	100
Phase O average	288							251		
Phase 1 average	24	0			5		29	0		73
Phase 2 average	1815	209	6924	659	334	993	28919	115	236	141
Phase 3 average	5	39	54	51	51		15	57		102

In both groups of patients (P1-P11 and P12-P76), a general pattern of infectivity was observed in the TCID assay for detecting infectious virus particles. At the beginning of HEP1 therapy, (Phase 0- Phase 1), the load of infectious HIV virus declined sharply to low levels within the first three weeks of treatment and in 9 out of 21

patients dropped to zero for at least one week. Three patients experienced an increase in infectivity during the first week of treatment before infectivity dropped to zero in the second week. In the second phase which followed, (Phase 2), in the majority of patients HIV infectivity rose between 2X and 600X between four and eight weeks after the start of treatment. The patients reported no worsening of their condition during this period. Phase 3 followed where infectivity declined to below pre-treatment levels.

In Phase 1, the average maximum decline of virus levels below the pre-treatment baseline was minus 80%. In Phase 2, the average maximum increase of virus in Phase Two was 22 times. In Phase 3, the average maximum decline of virus below the pre-treatment baseline was minus 64%. During Phase 3, viral infectivity declined to zero in three patients. Six months after the end of treatment with HEP1, viral infectivity generally returned to pre-treatment levels.

I interpret these results as showing that the immune system was activated by HEP1 to fight HIV in Phase One. The increased level of activation in the immune system stimulated activation of a reservoir of cells latently infected with HIV leading to the increase in infectious virus in Phase 2. Finally in Phase 3, the activated immune system successfully destroyed the newly activated virus reservoir. The group of ten patients treated for only one month with HEP1 showed that the progression through Phase 2 and Phase 3 did not depend on the presence of HEP1.

HIV viral load by quantitative plasma HIV RNA PCR

Analysis of viral load was performed by the Roche labs PCR assay: Amplicor HIV-1 Monitor.

Patient	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
HIV plasmaRNA in 1000s copies /ml	0.5	10	2	43	<0.4	10	94	22	10	21	<0.4
re-based to 100 at start	100	100	100	100	0	100	100	100	100	100	0
Phase O average						108	143		101		
Phase 1 average			38	39	28	37	68	78	90	45	
Phase 2 average	476	197	103	64	28	223	112	110	1535	92	
Phase 3 average		85	66	77	0	64	92	52		39	

Patient	P12	P17	P21	P63	P67	P68	P69	P72	P73	P76
HIV plasmaRNA in 1000s copies /ml	13	11	11	4	230	<0.4	8	25	<0.4	9
re-based to 100 at start	100	100	100	100	100	0	100	100	100	100
Phase O average										
Phase 1 average		0			78					55
Phase 2 average	219	177	273	206	91		142	191		139
Phase 3 average	135	86		98	63		138	72		72

In the majority of patients of both the one month and three month treatment, similar phases of viral suppression (Phase 1) followed by temporary viral activation (Phase 2) follow by suppression (Phase 3) were observed which was similar to the TCID data. Phase I lasted between one and four weeks and viral load declined on average by minus 47%. Phase 2 lasted between eight and forty weeks (a more sustained period than seen with the TCID assay) and viral RNA in the plasma increased on average by 3X. This was followed by Phase 3 in which viral RNA in the plasma fell below pre-treatment levels by an average reduction of minus 19%.

Cell populations and expression of cell surface markers by cytometry

Cells surface markers on peripheral blood mononuclear cells of the group of ten patients (P12-P76) treated for one month with HEP1 were analysed in detail using microscopy, fluorescent labelled antibodies, flow cytometry and related procedures. HEP1 treatment had the following effects on each cell populations averaged for the 10 patient group:

a) Lymphocytes

an average increase of 7 % in absolute number of lymphocytes during treatment and an increase of 25% for the five months following treatment.

b) Leucocytes

an average increase of 10% during treatment and an increase of approximately 20% for the five months following treatment.

d) Natural Killer Cells

An average increase of 10% during treatment and an increase of approximately 30% for the five months following treatment

e) B cells

An average increase of 5% during treatment and an increase of approximately 80% for the five months following treatment

f) CD3 expressing cells

An average increase of 15% during treatment and an increase of approximately 30% for the five months following treatment

g) CD8 expressing cells

An average increase of 15% during treatment and an increase of approximately 20% for the five months following treatment

h) CD44-CD4 expressing cells

An average increase of 25% during treatment and an increase of approximately 60% for the five months following treatment

h) CD44-CD8 expressing cells

An average increase of 12% during treatment and an increase of approximately 30% for the five months following treatment

i) HLA ClassI-CD4 expressing cells

An average increase of 10% during treatment and an increase of approximately 70% for the five months following treatment

j)HLA ClassI-CD8 expressing cells

An average increase of 10% during treatment and an increase of approximately 70% for the five months following treatment.

k) CD25-CD8 cells

An average increase of 5% during treatment from low levels and an increase of approximately 100% for the five months following treatment. CD25-CD4 cells did not show significant variation.

l) CD38-CD4 cells

An average increase of 2% during treatment from low levels and a decrease of approximately 15% for the five months following treatment.

m)CD38-CD8 cells

An average increase of 2% during treatment from low levels and a decrease of approximately 25% for the five months following treatment. (the significant decrease in CD38 is discussed in the next section)

Other markers such as CD28, HLA-DR, CD45RO,CD45RA, CD57,CD62L, showed no significant changes or consistent patterns between patients.

CD38-CD8 cells: prognostic indicator for progression to AIDS

It is well recognised that the increasing size of the population of cells expressing CD38-CD8 correlates with the development of AIDS in HIV infected patients.

M Levancher, F Hulstaert, S. Tallet, S Ullery, J J Pocidalo, B A Bach (1992)
"The significance of activation markers on CD8 lymphocytes in human immunodeficiency syndrome: staging and prognostic value" *Clinical Experimental Immunology* **90** 376-382

Briefly, the CD38-CD8 cells as a percent of the total CD8 population correlate with HIV disease progression, an observation which has been verified in a number of more recent publications. In healthy people, the percentage of the CD8 cells which also express CD38 is between 30-50%, in asymptomatic HIV infected patients is between 50-65%, in HIV infected patients with ARC is between 65% and 80% and in AIDS patients between 80%and 98%. In the follow up of the study of ten patients who took 10 mg HEP1 orally for one month, 5 patients provided blood samples for analysis. In all of these patients it was clear that the population of CD38-CD8 cells as a percent of the total CD8 population declined toward values indicating lower risk of HIV disease and improving health.

CD38-CD8 cells as a % CD8cells	Baseline	Month 1 treatment	Month 2	Month 3	Month 4	Month 5	Month 6
Patient 12	92	94		79	84	73	72
Patient 17	66	71		69	50	55	57
Patient 21	83	86				66	
Patient 69	90	94		86	79	71	
Patient 76	78	84		77	64	54	50

Anti-HIV antibodies in plasma

At the end of one month treatment with HEP1 of 10 patients (P12-P76), 5 patients showed significantly higher antibody titres against various HIV antigens (titres re-based to 100 before treatment).

	Anti gp120	Anti gp41	Anti-p31	Anti p24	Anti p17
Patient 17	216	93	157	97	101
Patient 21	127	110	71	103	110
Patient 63	110	131	387	146	154
Patient 69	160	274	813	507	116
Patient 72	93	111	147	245	143

Antibody responses to opportunistic infections

HEP1 treatment stimulated antibody responses to opportunistic infections (antibody titre re-based to 100 before treatment)

Max titre during treatment	P12	P17	P21	P63	P67	P68	P69	P72	P73	P76
Aspergillus IgG	122	97	132	116	89	114	119	190	142	111
Candida IgG	127	100	126	170	447	51	293	214	120	129
CMV IgG	290	81	227	186	118	91	105	112	152	88
CMV IgM	120	141	142	215	159	92	102	148	120	142
HSV1 IgM	113	129	158	134	99	89	116	101	107	106
HSV2 IgM	107	129	109	108	156	158	146	111	300	186
Toxoplasma IgG	105	107	98	108	125	101	104	95	105	98

The average increase in antibody titre was plus 23% for Aspergillus IgG, plus 78% for Candida IgG, plus 50% for CMV IgG, plus 38% for CMV IgM, plus 15% for HSV1 IgM, plus 51% for HSV2 IgM and plus 5% for Toxoplasma IgG.

Conclusion

The above data is consistent with the invention that a peptide with a sequence identical to part of the Hepreceptor leads to immune activation and clinical benefits in a human clinical trial of HIV patients.

EXAMPLE 2

Severe acute and chronic Candida infection in women can be cured by the immune response resulting from Hepreceptor stimulation.

Clinical Study: recurrent moderate candida infection

Female VJ (age 27) with an untreated fresh out-break of Candida infection volunteered for the study. She reported recurrent moderate vaginal Candida infection (six episodes in previous twelve months) which had been previously treated with intra-vaginal application of 1% clotrimazole. She self-administered 5ml of a 1mg/ml solution of HEP1 intra-vaginally with a 5ml syringe on two consecutive days. After three days all clinical symptoms of Candida infection had disappeared and she reported no further recurrences of Candida infection in the 12 month follow-up.

Clinical Study: severe persistent candida infection

Three female patients attending the Nearmedic STD clinical in Moscow volunteered for the study who were suffering from severe candida infection of the vagina after they had been treated with antibiotics for various genitourinary infections. The patients were treated with 5ml of a 2mg/ml solution of HEP1 for three consecutive days (no other antifungal treatment was used). Comparison of microbiological analysis (cultivation of urethral, cervical canal and vaginal swabs) and clinical analysis before treatment and three weeks after treatment demonstrated either significant improvement or elimination of the infection.

Patient	Age	Period	Clinical analysis	Urethra swab	Cervical swab	Vaginal swab
LLA	34	Before	Severe infection	Intense growth	Low growth	Low growth
KEM	28	Before	Severe infection	Intense growth	Intense growth	Intense growth
ALN	35	Before	Severe infection	Intense growth	Intense growth	Intense growth
LLA	34	After	No symptoms	Absent	Absent	Absent
KEM	28	After	mild symptoms	Low growth	few	Absent
ALN	35	After	No symptoms	Absent	Absent	Absent

EXAMPLE 3

Rupe312, Rupe414, Rupe111 and Rupe411 induce a strong macrophage activation response in mice.

A number of peptides derived from of the Hepreceptor Domain A including HEP1 were studied in mice.

Induction of activated macrophages

Groups of three mice (CDAxC57Bl)F1 weighing 22-24g were injected abdominally with each peptide solution of 1.0 microgram of peptide dissolved in 0.5ml of physiological saline. After 24 hours the animals were killed using neck vertebrae dislocation and 5 ml of Hanks solution was injected into the abdomen. The abdomen was massaged for 30 seconds and then the peritoneal liquid was collected. The collected liquid was filtered using a nylon filter into siliconised tubes containing 1.5mg/ml EDTA.

The number of nucleus containing cells in 1microgram of filtrate were then assessed under microscopic examination using a Nihon hemocytometer. The cells were pelleted by centrifugation for 5 minutes at 800g, the pellet was resuspended in fetal calf serum, the cell suspension was dropped on to a glass microscope slide and dried then fixed in methanol and stained with Romanovski's colouring agent. Morphological analysis of the cells of the peritoneal exsudate were performed using an Opton optical microscope at 1600 magnifications. The number of lymphocytes, resting macrophages, activated macrophages, granulocytes and other cell types were assessed. The result was that the peptides all increased the number of activated macrophages but Rupe111, Rupe312, Rupe411 and Rupe414 were significantly more active than HEP1.

1.0 microgram peptide /mouse	saline	HEP1	Rupe111	Rupe312	Rupe411	Rupe414
Activated macrophages as a percent of total number of cells	1.9	2.2	4.3	13.9	5.5	8.5

HEP1	SEQ ID 28	TEKKRRETVEREKE
Rupe111	SEQ ID 5:	TEKKRRETVER
Rupe312	SEQ ID 8:	KKRRETVERE
Rupe411	SEQ ID 11:	KRRETVER
Rupe414	SEQ ID 13:	KRRETVEREKE

EXAMPLE 4

The in vitro activation of human peripheral blood mononuclear cells by Hepreceptor peptides demonstrated by measuring the incorporation of radioactive tritiated thymidine into the DNA of growing cells.

Peripheral Blood Mononuclear Cells (PBMC) were separated from the peripheral blood of a healthy donor using the standard method of fractionation in a ficoll gradient. The PBMC were suspended in culture medium containing RPMI1640 medium plus 10% fetal calf serum, 1mM L-glutamin and antibiotics (BM). The cell suspension was placed in wells of a 96 hole microwell plate for cell cultivation, (100 microlitres of suspension containing 100,000 cells per well). Then 100 microlitres of BM was added containing peptide (final concentration 0.001-10 microgram/ml). The negative control well contained BM but no peptide. The plate was incubated at 37°C for three days then radioactive ^{3}H thymidine was added to a final concentration of 1 microcurie per ml. The incorporation of ^{3}H thymidine into the DNA of the cells was measured using a betacounter using standard procedures. The experiment was repeated twice and the results expressed as an average of the two experiments in radioactive counts per minute. The result showed that all the peptides activate mononuclear cell proliferation but that Rupe312 and Rupe414 were significantly more active than HEP1 with peak activity around 3 nanograms/ml.

Peptide microg/ml	Control	HEP1	Rupe19	Rupe312	Rupe414	Rupe411	Rupe111	Rupe614
0.0001	370	410	426	563	493	385	483	464
0.0003	370	500	602	742	580	510	483	503
0.001	370	989	718	976	684	702	710	550
0.003	**370**	**700**	**756**	**3222**	**2087**	**598**	**665**	**752**
0.01	370	628	545	656	650	532	537	607
0.03	370	517	586	539	596	500	642	538
0.1	370	456	537	533	485	499	633	596
0.3	370	399	563	611	492	486	668	635
1	370	400	509	472	449	468	529	600
3	370	412	502	455	437	420	486	499
10	370	501	517	405	394	390	470	412

HEP1	SEQ ID 28	TEKKRRETVEREKE
Rupe19	SEQ ID 4:	TEKKRRETV
Rupe312	SEQ ID 8:	KKRRETVERE
Rupe414	SEQ ID 13:	KRRETVEREKE,
Rupe411	SEQ ID 11:	KRRETVER
Rupe111	SEQ ID 5:	TEKKRRETVER
Rupe614	SEQ ID 18:	RETVEREKE

EXAMPLE 5

Effect of Hepreceptor peptides on expression of MHC Class I on various immunological cells

The incubation of Hepreceptor derived peptides (0.003 micrograms per ml) with human White Blood Cells (WBC) for 24 hours at 37°C, resulted in a fall in the intensity of HLA expression on the cell surface of all WBC (due to cell activation and receptor internalisation). Rupe 312 was more active than Rupe 414 which was more active than HEP1.

Data rebased to 100 for the control value in the absence of peptides

Density of cell surface expression of HLA Class I					
	Monocytes / Macrophages	CD8 lymphocytes	CD4 lymphocytes	B and NK cells	Granulocytes
Control	100	100	100	100	100
HEP1	83	83	80	84	89
Rupe414	83	76	77	77	85
Rupe312	71	72	70	73	81

A cell specific effect of this activation was an increase in the population of monocytes expressing MHC Class I and a decrease in the population of CD8 lymphocytes expressing MHC Class I.

Data rebased to 100 for the control value in the absence of peptides

Percentage of cell population expressing HLA Class I					
	Monocytes / Macrophages	CD8 lymphocytes	CD4 lymphocytes	B and NK cells	Granulocytes
Control	100	100	100	100	100
HEP1	108	91	105	102	100
Rupe414	109	89	105	101	98
Rupe312	119	85	104	105	98

EXAMPLE 6
Rupe312 suppresses IL-8 production in WBC

The suppressive effect of increasing concentrations of Rupe312 on the expression of IL-8 by human WBC after a 48 hour incubation at 37°C was detected. IL-8 is a chemotactic factor that is produced in response to inflammatory stimulus which attracts and activates T cells, neutrophils, basophils, granulocytes but not monocyte/macrophages. The inhibition of IL-8 may play a role in the selective activity of Rupe 312 in activating monocytes macrophages. The measurement of IL-8 provides an assay for determining the activity of various Hepreceptor derived peptides.

Rupe312 concentration in micrograms/ml	IL-8 concentration in culture in picrograms/ml
0	18900
0.001	13900
0.003	10700
0.01	8984
0.03	7869
0.1	6426

(IL-8 EIA assay manufactured by Innogenetics, Belgium)

SEQUENCES LISTING

GENERAL INFORMATION
NUMBER OF SEQUENCES: 28

INFORMATION FOR SEQ ID 1
SEQUENCE CHARACTERISTICS:
LENGTH: 32 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 1
Hepreceptor Domain A

A R E E K H Q K Q L E R Q Q L E T E K K R R E T V E R E K E Q M
1 5 10 15 20 25 30

INFORMATION FOR SEQ ID 2
SEQUENCE CHARACTERISTICS:
LENGTH: 34 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 2
Hepreceptor Domain B

M R E K E E L M L R L Q D $Y_{(p)}$ E E K T K K A E R E L S E Q I Q R A L Q
1 5 10 15 20 25 30

INFORMATION FOR SEQ ID 3
SEQUENCE CHARACTERISTICS:
LENGTH: 5 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 3
Rupe15: TEKKR
1 5

INFORMATION FOR SEQ ID 4
SEQUENCE CHARACTERISTICS:
LENGTH: 9 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 4
Rupe19: TEKKRRETV
1 5

INFORMATION FOR SEQ ID 5
SEQUENCE CHARACTERISTICS:
LENGTH: 11 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 5
Rupe111: TEKKRRETVER
1 5 10

INFORMATION FOR SEQ ID 6
SEQUENCE CHARACTERISTICS:
LENGTH: 5 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 6
Rupe37: KKRRE
1 5

INFORMATION FOR SEQ ID 7
SEQUENCE CHARACTERISTICS:
LENGTH: 8 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 7
Rupe310: KKRRETVE
1 5

INFORMATION FOR SEQ ID 8
SEQUENCE CHARACTERISTICS:
LENGTH: 10 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 8
Rupe312: KKRRETVERE
1 5 10

INFORMATION FOR SEQ ID 9
SEQUENCE CHARACTERISTICS:
LENGTH: 11 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 9
Rupe313: KKRRETVEREK
1 5 10

INFORMATION FOR SEQ ID 10
SEQUENCE CHARACTERISTICS:
LENGTH: 12 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 10
Rupe314: KKRRETVEREKE
1 5 10

INFORMATION FOR SEQ ID 11
SEQUENCE CHARACTERISTICS:
LENGTH: 8 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 11
Rupe411: KRRETVER
1 5

INFORMATION FOR SEQ ID 12
SEQUENCE CHARACTERISTICS:
LENGTH: 10 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 12
Rupe413: KRRETVEREK
1 5 10

INFORMATION FOR SEQ ID 13
SEQUENCE CHARACTERISTICS:
LENGTH: 11 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 13
Rupe414: KRRETVEREKE
1 5 10

INFORMATION FOR SEQ ID 14
SEQUENCE CHARACTERISTICS:
LENGTH: 5 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 14
Rupe59: RRETV
1 5

INFORMATION FOR SEQ ID 15
SEQUENCE CHARACTERISTICS:
LENGTH: 9 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 15
Rupe614: RETVEREKE
1 5

INFORMATION FOR SEQ ID 16
SEQUENCE CHARACTERISTICS:
LENGTH: 5 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 16
Rupe1014: EREKE
1 5

INFORMATION FOR SEQ ID 17
SEQUENCE CHARACTERISTICS:
LENGTH: 14 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 17
Rupe1024 EREKEQMMREKEEL
1 5 10

INFORMATION FOR SEQ ID 18
SEQUENCE CHARACTERISTICS:
LENGTH: 5 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 18
Rupe2024: KEELM
1 5

INFORMATION FOR SEQ ID 19
SEQUENCE CHARACTERISTICS:
LENGTH: 13 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 19
Rupe2032: KEELMLRLQDYEE
1 5 10

INFORMATION FOR SEQ ID 20
SEQUENCE CHARACTERISTICS:
LENGTH: 13 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 20
Rupe2032p: KEELMLRLQDYpEE
1 5 10

INFORMATION FOR SEQ ID 21
SEQUENCE CHARACTERISTICS:
LENGTH: 12 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 21
Rupe2132: EELMLRLQDYEE
1 5 10

INFORMATION FOR SEQ ID 22
SEQUENCE CHARACTERISTICS:
LENGTH: 12 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 22
Rupe2132p: EELMLRLQDYpEE
1 5 10

INFORMATION FOR SEQ ID 23
SEQUENCE CHARACTERISTICS:
LENGTH: 11 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 23
Rupe2232: ELMLRLQDYEE
1 5 10

INFORMATION FOR SEQ ID 24
SEQUENCE CHARACTERISTICS:
LENGTH: 11 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 24
Rupe2232p: ELMLRLQDYpEE
1 5 10

INFORMATION FOR SEQ ID 25
SEQUENCE CHARACTERISTICS:
LENGTH: 5 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 25
Rupe2428: MLRLQ
1 5

INFORMATION FOR SEQ ID 26
SEQUENCE CHARACTERISTICS:
LENGTH: 5 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 26
Rupe2832: QDYEE
1 5

INFORMATION FOR SEQ ID 27
SEQUENCE CHARACTERISTICS:
LENGTH: 5 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION
SEQ ID 27
Rupe2832p: QDYpEE
1 5

INFORMATION FOR SEQ ID 28

SEQUENCE CHARACTERISTICS:

LENGTH:	14 amino acids
TYPE:	amino acid
STRANDEDNESS:	single
TOPOLOGY:	linear
MOLECULAR TYPE:	peptide

SEQUENCE DESCRIPTION

SEQ ID 28

HEP1: TEKKRRETVEREKE

1 5 10

CLAIMS

1. a charged molecule that binds to the Hepreceptor with greater affinity than HEP1.
2. a charged molecule of claim 1, where such charged molecule is a peptide
3. a peptide of claim 2, where such peptide is between five and thirteen amino acids in length has a sequence identical to part of the Hepreceptor
4. a peptide consisting of the amino acid sequence;
A R E E K H Q K Q L E R Q Q L E T E K K R R E T V E R E K E Q M
5. a peptide consisting of the amino acid sequence;
M R E K E E L M L R L Q D Y(p) E E K T K K A E R E L S E Q I Q R A L Q
6. a peptide consisting of the amino acid sequence;
TEKKR
7. a peptide consisting of the amino acid sequence;
TEKKRRETV
8. a peptide consisting of the amino acid sequence;
TEKKRRETVER
9. a peptide consisting of the amino acid sequence;
KKRRE
10. a peptide consisting of the amino acid sequence;
KKRRETVE
11. a peptide consisting of the amino acid sequence;
KKRRETVERE
12. a peptide consisting of the amino acid sequence;
KKRRETVEREK
13. a peptide consisting of the amino acid sequence;
KKRRETVEREKE
14. a peptide consisting of the amino acid sequence;
KRRETVER
15. a peptide consisting of the amino acid sequence;
KRRETVEREK
16. a peptide consisting of the amino acid sequence;
KRRETVEREKE
17. a peptide consisting of the amino acid sequence;
RRETV
18. a peptide consisting of the amino acid sequence;
RETVEREKE
19. a peptide consisting of the amino acid sequence;
EREKE
20. a peptide consisting of the amino acid sequence;
EREKEQMMREKEEL
21. a peptide consisting of the amino acid sequence;
KEELM
22. a peptide consisting of the amino acid sequence;
KEELMLRLQDYEE
23. a peptide consisting of the amino acid sequence;
KEELMLRLQDYpEE
24. a peptide consisting of the amino acid sequence;
EELMLRLQDYEE
25. a peptide consisting of the amino acid sequence;
EELMLRLQDYpEE

26. a peptide consisting of the amino acid sequence;
ELMLRLQDYEE
27. a peptide consisting of the amino acid sequence;
ELMLRLQDYpEE
28. a peptide consisting of the amino acid sequence;
MLRLQ
29. a peptide consisting of the amino acid sequence;
QDYEE
30. a peptide consisting of the amino acid sequence;
QDYpEE

ABSTRACT

This invention describes novel charged molecules which specifically bind to the Hepreceptor, a regulatory site which I have discovered in human ezrin. My invention is that when peptides or other charged molecules bind to the Hepreceptor, medically useful immune responses are induced. These charged molecules can be administered orally and by other routes for the treatment of various infectious diseases and cancer.

I have determined that the Hepreceptor (human ezrin 308-373) comprises of two adjacent alpha helical domains which are folded together at a hinge region (M339-M340) and stabilised by complimentary side chain charges of the primary amino acid sequence in the soluble cytoplasmic conformation of ezrin. I have determined that in the unfolded membrane associated conformation of ezrin, the Hepreceptor is pushed through the cell membrane and is exposed on the outer surface of the cell. Hepreceptor-Domain A (amino acid numbers 308-339 of human ezrin), comprises of the following 32 amino acid sequence.
SEQ ID 1
A R E E K H Q K Q L E R Q Q L E T E K K R R E T V E R E K E Q M
Hepreceptor-Domain B (amino acid numbers 340-373 of human ezrin), comprises of the following 34 amino acid sequence (Tyrosine 353 [Y] may be phosphorylated to phosphotyrosine [Yp] in the membrane associated conformation of ezrin):
SEQ ID 2
M R E K E E L M L R L Q D Y(p) E E K T K K A E R E L S E Q I Q R A L Q

I have designed novel charged peptides which have sequences identical to the amino acid sequences of the complementary domains of the Hepreceptor which can bind and activate the Hepreceptor. These peptides, which are a subject of this invention, bind to the cell surface Hepreceptors and stabilise the unfolded conformation of ezrin and induce immuno-modulatory effects. The preferred peptides are between five and thirteen aminoacids in length and the preferred sequences are as follows:

(The sequences are listed using the single letter code for each amino acid written from the N terminus to the C terminus of the polypeptide. Yp represents the form of phospho-tyrosine found in vivo)

SEQ ID 3, Rupe15:	TEKKR
SEQ ID 4, Rupe19:	TEKKRRETV
SEQ ID 5, Rupe111:	TEKKRRETVER
SEQ ID 6, Rupe37:	KKRRE
SEQ ID 7, Rupe310:	KKRRETVE
SEQ ID 8, Rupe312:	KKRRETVERE
SEQ ID 9, Rupe313:	KKRRETVEREK
SEQ ID 10, Rupe314:	KKRRETVEREKE
SEQ ID 11, Rupe411:	KRRETVER
SEQ ID 12, Rupe413:	KRRETVEREK
SEQ ID 13, Rupe414:	KRRETVEREKE
SEQ ID 14, Rupe59:	RRETV
SEQ ID 15, Rupe614:	RETVEREKE
SEQ ID 16, Rupe1014:	EREKE
SEQ ID 17, Rupe1024	EREKEQMMREKEEL
SEQ ID 18, Rupe2024:	KEELM
SEQ ID 19, Rupe2032:	KEELMLRLQDYEE

SEQ ID 20, Rupe2032p:	KEELMLRLQDYpEE
SEQ ID 21, Rupe2132:	EELMLRLQDYEE
SEQ ID 22, Rupe2132p:	EELMLRLQDYpEE
SEQ ID 23, Rupe2232:	ELMLRLQDYEE
SEQ ID 24, Rupe2232p:	ELMLRLQDYpEE
SEQ ID 25, Rupe2428:	MLRLQ
SEQ ID 26, Rupe2832:	QDYEE
SEQ ID 27, Rupe2832p:	QDYpEE

FIGURE 1

Primary/secondary structure of the Hepreceptor

Note how the highly charged region matches positive to negative charges when either binding to itself, to a synthetic ligand such as Rupe312, or binding to another hepreceptor.

Figure 1a: Inactive FOLDED conformation of the Hepreceptor comprising of a pair of associated anti-parallel alpha helices internally located in soluble cytoplasmic ezrin
(Note tyrosine 353 is not phosphorylated)

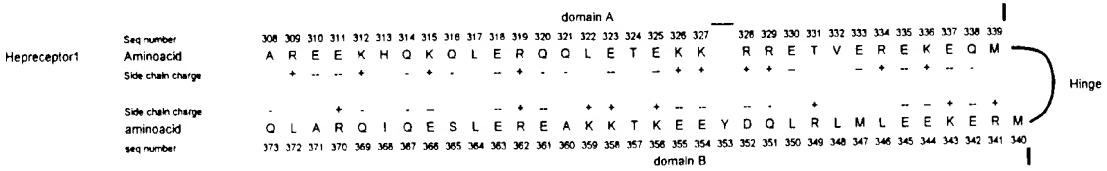


Figure 1b: Active OPEN conformation of the Hepreceptor located in membrane associated ezrin on the external surface of the cell membrane binding to Rupe312, an example of a synthetic ligand
(In the active conformation tyrosine 353 is phosphorylated)

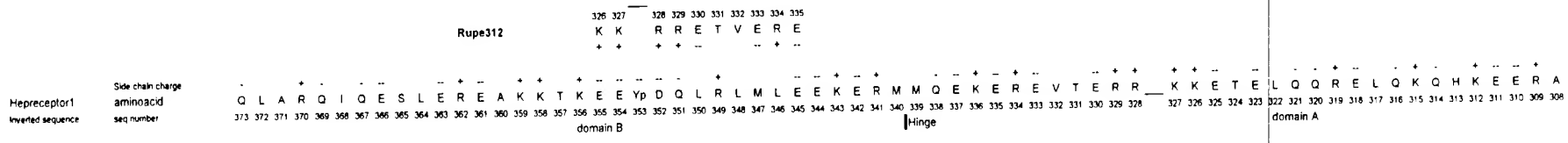
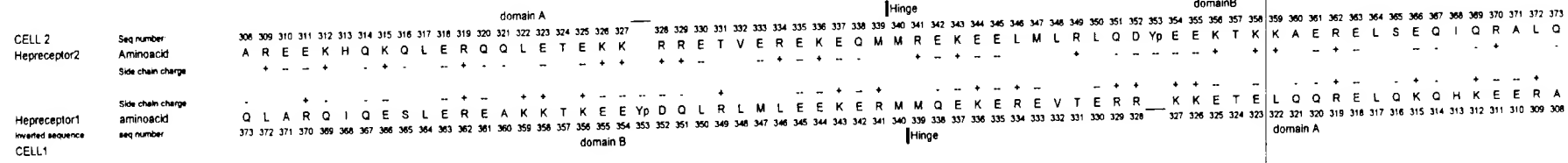


Figure 1c: Dimerisation between two Hepreceptors in the active OPEN conformation for example, located in membrane associated ezrin on the external surface of the cell membrane binding to a Hepreceptor on another cell
(Note the double length pair of anti-parallel alpha helices)
(In the active conformation tyrosine 353 is phosphorylated)



1/2

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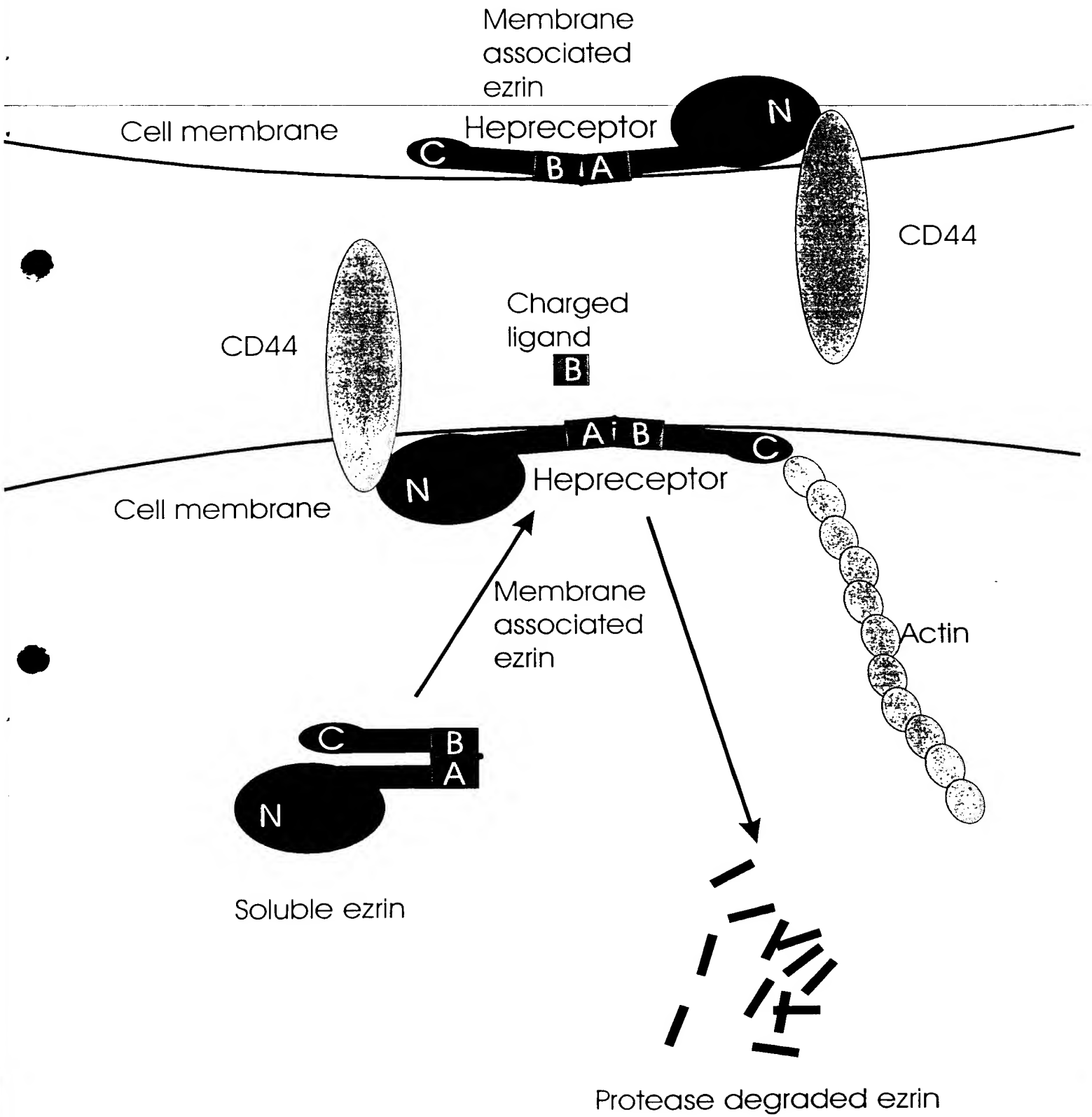
FIGURE 2

Cellular locations and complexes of ezrin



Holms R.D

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(P. Treen)